

CLAIMS

1. A method for the quantitative evaluation of a rearrangement or of a targeted genetic recombination of 5 an individual, which method is characterized in that it comprises at least:

(a) the extraction of human genomic DNA from a biological sample,

10 DNA, between a few hundred base pairs and several tens of kb in size, by multiplex PCR, in the presence:

* of one or more pairs of primers, selected so as to correspond to the following characteristics:

15 - at least one of said primers of one of said pairs of primers hybridizes upstream and/or at the 5' end of a Vx gene to be amplified, which may be involved in said genetic rearrangement;

20 - at least the other of said primers of one of said pairs of primers hybridizes downstream and/or at the 3' end of a Jy gene to be amplified, which may be involved in said genetic rearrangement;

25 * and of a DNA polymerase or a mixture of DNA polymerases for amplifying genomic DNA segments between a few hundred base pairs and several tens of kb in size, preferably greater than 10 kb in size, and having a correction activity that makes it possible to substantially improve the elongation;

30 said amplification comprising, in addition to the initial denaturation step, cycles of denaturation, hybridization and elongation, in which the elongation steps are carried out at least for 10 minutes at 68°C-72°C;

35 c) the separation of the gDNA fragments amplified, and

d) the detection of the rearranged or recombined segments.

2. The method for the quantitative evaluation of the

immune repertoire of an individual by genetic rearrangement as claimed in claim 1, characterized in that it comprises:

5 a) the extraction of human genomic DNA from a biological sample,

b) the amplification of a segment of said genomic DNA, between a few hundred base pairs and several tens of kb in size, by multiplex long PCR, in the presence:

10 * of one or more pairs of primers, selected so as to correspond to the following characteristics:

15 - at least one of said primers of one of said pairs of primers, called primer V, hybridizes specifically with a region located upstream of the RSS sequence of a V_x gene to be amplified, corresponding to a V segment of the variable domain of the α chain of a T-cell receptor (TCRAD);

20 - at least one of said primers of one of said pairs of primers, called primer J, hybridizes specifically with a region located downstream of the RSS sequence of a J_y gene to be amplified, with the 3' end of said J_y gene to be amplified or in said J_y gene to be amplified, corresponding to a J segment of the α chain of a T-cell receptor;

25 * and of a DNA polymerase or a mixture of DNA polymerases for amplifying genomic DNA segments between a few hundred base pairs and several tens of kb in size, and having a correction activity that makes it possible to substantially improve the elongation;

30 said amplification comprising, in addition to the initial denaturation step, cycles of denaturation, hybridization and elongation, in which the elongation steps are carried out at least for 10 minutes at 68°C-72°C;

35 (c) the separation of the gDNA fragments amplified, and

(d) the detection of the recombined V(D)J segments.

3. The method as claimed in claim 1 or claim 2,

characterized in that, in the amplification step (b), the selection of the primers is carried out:

- by systematic analysis of the entire locus concerned, and in particular of the human TCRAD locus,
5 using a suitable software,
- selection of the primers whose 3'OH end is complementary only to the region of interest,
- elimination of the primers forming autodimers or stable hairpins, in particular by analysis with a
10 suitable software, and
- elimination of the pairs of primers which form hybrids with one another.

4. The method as claimed in claim 3, characterized in
15 that the primers V and J of the pairs of primers V/J are selected from the group consisting of the primers of sequences SEQ ID NO: 1-21.

5. The method as claimed in any one of claims 1 to 4,
20 characterized in that the amplification step (b) advantageously uses additional primers for amplifying, in addition, at least one of the following segments: D segments, V segments and J segments of the TCR β , γ , δ chains and, optionally, segments of the immunoglobulin
25 chains.

6. The method as claimed in any one of claims 1 to 5, characterized in that, in the amplification step (b), the multiplex long PCR (LPCR) reaction is carried out
30 after purification of the DNA, or directly on a cell lysate.

7. The method as claimed in any one of claims 1 to 6, characterized in that, in the amplification step (b),
35 the elongation steps are incremented by 15-20 seconds per additional elongation cycle.

8. The method as claimed in any one of claims 1 to 7, characterized in that step (c) consisting of separation

of the amplified DNA fragments is carried out by electrophoretic migration on a gel, preferably pulsed-field migration.

5 9. The method as claimed in any one of claims 1 to 7, characterized in that step (c) consisting of separation of the amplified DNA fragments is carried out by microcapillary separation.

10 10. The method as claimed in any one of claims 1 to 9, characterized in that the detection step (d) can advantageously be carried out by Southern transfer of the amplified products onto nylon membranes, followed by visualization after hybridization with one or more 15 nucleotide probes labeled with a radioactive isotope or a fluorochrome.

11. The method as claimed in claim 10, characterized in that the probes are advantageously selected from the 20 group consisting of the sequences SEQ ID NO: 22-37.

12. The method as claimed in any one of claims 1 to 9, characterized in that the detection step (d) can advantageously be carried out by using a labeled base 25 (labeled with a radioactive isotope or a fluorochrome) during the amplification, and then by measuring the incorporation thereof directly in the gel.

13. The method as claimed in any one of claims 1 to 9, 30 characterized in that the detection step (d) can advantageously be carried out by using a DNA-labeling agent during the migration, and detecting after excitation in the UV range or at another appropriate wavelength.

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14. The method as claimed in any one of claims 1 to 9, characterized in that the detection step (d) can advantageously be carried out by using primers labeled with fluorochromes or other enzymatic revealing means

during the amplification.

15. A method for the follow-up to a treatment for a pathology in which the immune repertoire is initially modified, in an individual concerned, which method is characterized:

- in that it implements the method for the evaluation of the immune repertoire, as claimed in any one of claims 1 to 14, at the beginning of treatment,

10 - in that said evaluation method is reiterated at various phases of the treatment, and

- in that the profile of the immune repertoire obtained each time is compared with that of a standard immune repertoire, in order to evaluate the response of 15 said individual to said treatment.

16. A method for the measurement of the antigen receptor repertoire during the various phases of a pathology in which the immune repertoire is modified, 20 in an individual concerned, which method is characterized:

- in that it implements the method for the evaluation of the immune repertoire, as claimed in any one of claims 1 to 14, at various phases of the 25 pathology, and

- in that the profile of the immune repertoire obtained each time is compared with that of a standard immune repertoire, in order to evaluate the evolution of said pathology.

30 17. The method as claimed in any one of claims 1 to 16, characterized in that the biological sample consists of T lymphocytes of any origin.

35 18. The method as claimed in claim 17, characterized in that said T lymphocytes are selected from the group consisting of thymic cells, of T lymphocytes from peripheral blood, of T lymphocytes from other lymphoid organs, of T lymphocytes from various organs and of T

lymphocytes derived from tumors or from inflammatory sites.

5 19. A kit for the quantitative evaluation of the immune repertoire of an individual, characterized in that it comprises, in addition to the usual buffers and reagents for carrying out a PCR, primers and probes as defined in claims 4 and 11.

10 20. A primer that can be used in a method as claimed in any one of claims 1 to 18, characterized in that it is selected from the group consisting of the oligonucleotide primers corresponding to the sequences SEQ ID NO: 1-21.

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21. A detection probe that can be used in a method as claimed in any one of claims 1 to 18, characterized in that it is selected from the group consisting of the oligonucleotide probes of sequences SEQ ID NO: 22-37.

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22. The use of the amplification primers and of the detection probes as claimed in claims 20 and 21, for the quantitative evaluation of the immune repertoire of an individual.